

Identification of human cyclin-dependent kinase 8, a putative protein kinase partner for cyclin C

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ABSTRACT Metazoan cyclin C was originally isolated by virtue of its ability to rescue *Saccharomyces cerevisiae* cells deficient in G₁ cyclin function. This suggested that cyclin C might play a role in cell cycle control, but progress toward understanding the function of this cyclin has been hampered by the lack of information on a potential kinase partner. Here we report the identification of a human protein kinase, K35 [cyclin-dependent kinase 8 (CDK8)], that is likely to be a physiological partner of cyclin C. A specific interaction between K35 and cyclin C could be demonstrated after translocation of CDKs and cyclins *in vitro*. Furthermore, cyclin C could be detected in K35 immunoprecipitates prepared from HeLa cells, indicating that the two proteins form a complex also *in vivo*. The K35–cyclin C complex is structurally related to SRB10–SRB11, a CDK–cyclin pair recently shown to be part of the RNA polymerase II holoenzyme of *S. cerevisiae*. Hence, we propose that human K35 (CDK8)–cyclin C might be functionally associated with the mammalian transcription apparatus, perhaps involved in relaying growth-regulatory signals.

Complexes between cyclin-dependent kinases (CDKs) and cyclin regulatory subunits play a pivotal role in cell cycle regulation in all eukaryotes (1, 2). In the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, cell cycle progression is controlled predominantly by a single CDK, termed p34^{CDK2} and p34^{cdk2}, respectively (3–5). In metazoans, however, different cell cycle transitions require distinct CDKs (6–8). Cyclins are positive regulatory subunits of CDKs and constitute multiprotein families in yeasts and metazoans (9, 10). Although the importance of many CDKs and cyclins in promoting the transitions between successive stages of the cell cycle is well established, it should not be assumed that all CDK–cyclin complexes function directly or exclusively in cell cycle control. This is illustrated best by studies on *S. cerevisiae*, where at least three distinct CDK–cyclin pairs, PHO85–PHO80, KIN28–CCL1, and SRB10–SRB11, have been implicated in the regulation of transcriptional events (refs. 11–13; for review, see ref. 14). PHO85 also functions in association with two additional cyclins, PCL1 and PCL2 (also termed HCS26 and ORF-D, respectively), possibly to integrate cell cycle progression with the availability of nutrients (15, 16). In vertebrates, six of seven presently known CDKs have been implicated in cell cycle progression, but CDK5 may function primarily as a neurofilament kinase in postmitotic neurons (17–20). Furthermore, the precise physiological role of the CDK7–cyclin H complex remains to be understood. Originally identified as a CDK-activating kinase (21–23), the CDK7–cyclin H complex was shown to form part of the general transcription factor TFIID and to display kinase activity toward the C-terminal domain of RNA polymerase II (24–26),

suggesting that it may also play a role in transcription and/or DNA repair.

There is no doubt that additional vertebrate CDKs and cyclins await discovery. In particular, a CDK partner has not yet been identified for metazoan cyclin C. This cyclin was originally isolated from human and *Drosophila* cDNA libraries by virtue of its ability to complement an *S. cerevisiae* strain lacking the G₁ cyclins CLN1–3 (27–29). The same complementation approach had yielded cDNAs for human cyclins D1 and E (27), both of which were subsequently shown to play important roles during the G₁/S phase transition of the metazoan cell cycle (10). The possibility has been considered that cyclin C might also represent a G₁ cyclin, but no support for this notion has yet been obtained. For cyclins D1 and E, the identification of CDK partners proved to be essential for studying the function of the respective CDK–cyclin complexes. Thus, progress toward understanding the function of cyclin C is expected to critically depend on the identification of a kinase complex partner.

Here we report the isolation of a human protein kinase, K35,[‡] that readily forms specific complexes with cyclin C *in vitro*. We furthermore show that cyclin C can be detected in immunoprecipitates of K35 prepared from metabolically labeled HeLa cells, indicating that the two proteins also form a complex *in vivo*. Hence, K35 is a bona fide CDK, which we propose to name CDK8. Although CDK8 is only distantly related to other mammalian CDKs, it shows a striking sequence similarity to SRB10 of *S. cerevisiae*. This observation raises the tantalizing prospect that metazoan CDK8–cyclin C functionally resembles the budding yeast SRB10–SRB11 complex and also plays an important role in the regulation of transcription.

MATERIALS AND METHODS

Cloning and Sequencing of a cDNA for K35 (CDK8). A 216-bp cDNA fragment spanning subdomains V1b–IX of K35 was obtained by the PCR (30) and used for screening of a human testis Agt11 library (Clontech). After plaque purification of phages, inserts were excised and subcloned into Bluescript vectors. The longest insert (1772 bp) was sequenced in both orientations and found to encode the entire K35 protein. Colony hybridization, phage isolation, subcloning, and plasmid sequencing were performed as described (31, 32).

Production of Anti-K35 Antibodies and Immunochromatological Techniques. Rabbit anti-K35 antibodies were produced and affinity-purified by standard methods (31, 33). The characterization of antibodies specific for cyclin C will be reported elsewhere (P.L., unpublished data). Western blot analysis was

Abbreviation: CDK, cyclin-dependent kinase.

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[‡]The sequence reported in this paper has been deposited in the GenBank data base [accession no. X85753 for K35 (CDK8)].

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FIG. 1. Nucleotide and deduced amino acid sequence of K35 (CDK8). The 1772-bp K35 cDNA codes for a protein of 464 amino acids. The putative initiator ATG codon is in a suitable context for translation initiation, and *in vitro* translation of this cDNA yields a protein that comigrates exactly with immunoreactive HeLa cell K35 protein (see Fig. 3a). Amino acid residues highly conserved among protein serine/threonine kinases (36) are marked with solid circles. Boldface type denotes a motif, SACRE, in the region corresponding to PSTAIRE in CDC2 (37). We note that a DNA fragment corresponding to K35 has been isolated as part of a gene mapping project, indicating that K35 (CDK8) maps to human chromosome 13q12 (GenBank accession no. L23208). This locus is associated with several human disease genes, including the breast cancer susceptibility gene *BRCA2* (38).

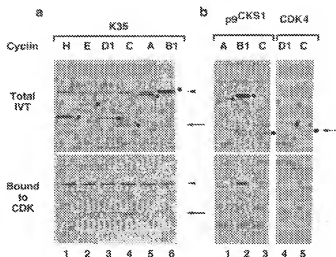


FIG. 2. Specificity of *in vitro* interaction between K35 and cyclin C. (a) Demonstration that K35 binds specifically to cyclin C. K35 and human cyclins A, B1, C, D1, E, and H were translated in a rabbit reticulocyte lysate in the presence of [³⁵S]methionine/cysteine (Express³⁵S, DuPont/NEN) and then mixed as indicated. One aliquot of each sample was analyzed directly by SDS/PAGE and fluorography (Upper). Total IVT, total *in vitro* translation. The remaining samples were subjected to immunoprecipitation (Lower) by using anti-K35 antibodies. Translated cyclins are marked by dots, and the positions of cyclin C and K35 are indicated by arrows and arrowheads, respectively. (b) Demonstration that cyclin C does not interact with CDK complex partners of cyclins A, B1, and D1. Cyclins A, B1, C, and D1 were translated in the presence of [³⁵S]methionine/cysteine (Upper). Their ability to interact with endogenous rabbit reticulocyte lysates (lanes 1–3) or unlabeled translated CDK4 (lanes 4 and 5) was then tested by isolation of CDK–cyclin complexes (Lower). Rabbit reticulocyte CDC2 and/or CDK2 were isolated with p9^{CKS1} affinity beads (lanes 1–3) and CDK4 was immunoprecipitated with anti-CDK4 antibodies (lanes 4 and 5). Translated cyclins are marked by dots, and the position of cyclin C is indicated by an arrow.

protein contains all the sequence motifs and 11 subdomains characteristic of a serine/threonine-specific kinase (36).

To examine the possibility that K35 might associate with a cyclin, K35 and human cyclins A, B1, C, D1, E, and H were produced by coupled *in vitro* transcription–translation. K35 was then incubated separately with each cyclin, and one part of each sample was resolved by SDS/PAGE (Fig. 2a Upper),

whereas the remainder was subjected to immunoprecipitation with anti-K35 antibodies (Fig. 2a Lower). Among all cyclins tested, only cyclin C coprecipitated reproducibly with K35 (lane 4). To determine whether cyclin C might be recognized directly by anti-K35 antibodies, the same type of experiment was performed with myc-epitope-tagged K35, by using anti-myc monoclonal antibodies for immunoprecipitation, with identical results (data not shown). Having established that K35 binds selectively to cyclin C, it was important to confirm the specificity of cyclin C for K35. We found that cyclin C bound to K35 but not to CDC2, CDK2, CDK4, or CDK7 (Fig. 2b and data not shown). In a first experiment, p9^{CKS1} affinity beads were used for isolating complexes formed between ³⁵S-labeled *in vitro*-translated cyclins and endogenous CDKs present in the reticulocyte lysate. Consistent with previous results (31), cyclins A and B1 could readily be recovered on p9^{CKS1} beads, reflecting their affinities for CDC2 and/or CDK2 (Fig. 2b, lanes 1 and 2). In contrast, cyclin C did not bind to p9^{CKS1} beads (Fig. 2b, lane 3), indicating that cyclin C did not interact with the complex partners of A- or B-type cyclins. To extend this analysis to CDK4, one of the CDKs that cannot be isolated by p9^{CKS1} (39), ³⁵S-labeled cyclins C and D1 were incubated with unlabeled *in vitro*-translated CDK4, and CDK4 was then immunoprecipitated with anti-CDK4 antibodies. (The reason for not labeling CDK4 in these experiments was that CDK4 comigrated almost exactly with cyclin C in our gel system.) Whereas cyclin D1 bound to CDK4, as expected (39), cyclin C did not (Fig. 2b, lanes 4 and 5). Thus, these studies demonstrate that K35 and cyclin C form a highly specific complex *in vitro*.

None of the available anti-cyclin C antibodies allowed us to immunoprecipitate cyclin C from cultured human cells efficiently (data not shown). To determine whether K35 and cyclin C interact *in vivo*, antibodies were raised against recombinant K35 and used for coimmunoprecipitation experiments. When assayed by Western blot analysis on total HeLa cell extracts, these antibodies recognized a single protein that comigrated exactly with *in vitro*-translated K35 (Fig. 3a, compare lanes 2 and 3), whereas a myc-epitope-tagged version of K35 displayed the expected reduced electrophoretic mobility (Fig. 3a, lane 1). When the same antibodies were used to immunoprecipitate K35 from ³⁵S-labeled HeLa cells, they precipitated a number of cellular proteins (Fig. 3b, lane 2), many of which were not precipitated by the corresponding preimmune serum (Fig. 3b, lane 3), suggesting that K35 might form part of a multiprotein complex. Of particular interest, one of the proteins coprecipitating

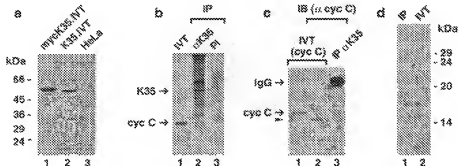


FIG. 3. K35 interacts with cyclin C *in vivo*. (a) Specificity of anti-K35 antibodies. Affinity-purified rabbit anti-K35 antibodies recognize a single 53-kDa protein in a lysate of total HeLa cell proteins (lane 3). This protein comigrates exactly with *in vitro*-translated K35 protein (lane 2). A myc-epitope-tagged *in vitro*-translated K35 displays slightly retarded electrophoretic mobility, as expected (lane 1). (b) Immunoprecipitation of K35-associated proteins from exponentially growing HeLa cells. Lanes: 1, *in vitro*-translated K35 and cyclin C as markers; 2 and 3, ³⁵S-labeled cellular proteins immunoprecipitated by anti-K35 serum and preimmune serum, respectively. (c) Immunological identification of cyclin C in K35 immune complexes. An immunoprecipitate (IP) was prepared from unlabeled HeLa cells, by using anti-K35 antibodies. Immune complexes (lane 3) and *in vitro*-translated cyclin C (lane 2) were subjected to immunoblot analysis (IB) with an anti-cyclin C antibody. To provide a marker for the migration of cyclin C, the same gel was also subjected to fluorography, allowing the detection of the ³⁵S-labeled *in vitro*-translation product (lane 1). We note that anti-cyclin C antibodies cross-reacted with an unidentified protein present in reticulocyte lysates (marked by small arrowhead). (d) Comparison of V8 protease digestion patterns obtained from ³⁵S-labeled *in vitro*-translated cyclin C (lane 2) and the 30-kDa protein coprecipitating with K35 from HeLa cells (lane 1).

brate CDC2 (34), it is interesting that both K35 and SRB10 contain phosphorylatable residues corresponding to Thr-14 and Tyr-15 in subdomain I but lack a threonine phosphorylation site in the exact same position of Thr-161 in the T-loop between subdomains VII and VIII (44, 47). Hence, K35 and/or SRB10 might be negatively regulated via phosphorylation of residues within the GXGXXG motif. Remarkably, however, they might not need to be phosphorylated by a CDK-activating kinase.

The SRB10 kinase and its associated cyclin subunit SRB11 form part of the RNA polymerase II holoenzyme of *S. cerevisiae* (13). Genetic studies implicate the SRB10/SRB11 gene products in both positive and negative regulation of transcription, particularly in *GAL* gene expression and glucose repression (refs. 13, 14, and 45 and D. Balciunas and H. Ronne, personal communication). How this yeast CDK-cyclin pair receives extracellular signals and relays them to the transcription apparatus is unknown, but available evidence indicates that the SRB10-SRB11 kinase is involved, either directly or indirectly, in phosphorylating the C-terminal domain of RNA polymerase II (13).

Could CDK8-cyclin C be a mammalian homologue of yeast SRB10-SRB11? A biochemical approach to answering this question is not yet possible since no mammalian homologue of the yeast RNA polymerase II holoenzyme has yet been purified. Our attempts at functionally complementing disruptions of the *SRB10* and *SRB11* genes by expressing human CDK8-cyclin C in yeast have so far been unsuccessful (D. Balciunas, J.-P.T., M.J., E.A.N., and H. Ronne, unpublished results). Nevertheless, the structural similarity between CDK8-cyclin C and SRB10-SRB11 suggests that the two CDK-cyclin pairs might perform related functions. If this hypothesis is correct, the original cloning of cyclin C by complementation might reflect the ability of cyclin C to induce the transcription of yeast cyclin genes rather than a direct activation of the CDC28 protein kinase. In any event, extrapolating from the genetic data obtained for SRB10-SRB11, we propose that CDK8-cyclin C might interact with the mammalian transcription apparatus and thereby contribute to integrate cell growth with cell cycle progression. The identification of a catalytic partner for cyclin C sets the stage for testing this hypothesis and for unraveling the function of this hitherto mysterious cyclin.

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